High Prevalence of Long QT Syndrome Associated SCN5A Variants in Patients with Early-Onset Lone Atrial Fibrillation

Running title: Olesen et al.; Nav1.5 mutations in lone AF

Morten S. Olesen, MSc, PhD1,2,3*; Lei Yuan, MD1,4*; Bo Liang MD, PhD1,4; Anders G. Holst, MD1,2,3; Nikolaj Nielsen, MSc1,4; Jonas B. Nielsen, MD1,2,4; Paula L. Hedley, MSc6; Michael Christiansen, MD5; Søren-Peter Olesen, MD, DMSc1,4; Stig Haunso, MD, DMSc1,2,3,5; Nicole Schmitt, MSc, PhD1,4; Thomas Jespersen, MSc, PhD, DMSc1,4; Jesper H. Svendsen, MD, DMSc, FESC1,2,3,5

1Danish National Research Foundation Centre for Cardiac Arrhythmia (DARC); 2Laboratory for Molecular Cardiology, The Heart Centre, Rigshospitalet; 3Dept of Cardiology, The Heart Centre, Rigshospitalet; 4Dept of Biomedical Sciences, Faculty of Health & Medical Sciences; 5Dept of Medicine & Surgery, Faculty of Health & Medical Sciences, University of Copenhagen; 6Dept of Clinical Biochemistry & Immunology, Statens Serum Institut, Copenhagen, Denmark

*Contributed equally

Address for correspondence:
Morten Olesen, PhD, MSc,
Laboratory for Molecular Cardiology
Department of Cardiology, Rigshospitalet
Juliane Mariesvej 20
2100 Copenhagen Ø, Denmark
Tel: (+45)35456506
Fax (+45)35456500
E-mail: morten.salling.olesen@rh.regionh.dk

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Abstract:

**Background** - Atrial fibrillation (AF) is the most common cardiac arrhythmia. The cardiac sodium channel, Na\(_v\)1.5, plays a pivotal role in setting the conduction velocity as well as the initial depolarization of the cardiac myocytes. We hypothesized that early-onset lone AF was associated with genetic variation in SCN5A.

**Methods and Results** - The coding sequence of SCN5A was sequenced in 192 early-onset lone AF patients. Eight non-synonymous mutations (T220I, R340Q, T1304M, F1596I, R1626H, D1819N, R1897W and V1951M) and two rare variants (S216L in two patients and F2004L) were identified. Six out of eleven geno-positive probands (3.2% of the total population) had a variant previously associated with long QT syndrome type 3 (LQTS3). The prevalence of LQTS3 associated variants in the lone AF patients was much higher than expected as compared to the prevalence in recent exome data (minor allele frequency 1.6% vs. 0.3%, P=0.003), mainly representing the general population. The functional effects of the mutations were analyzed by whole-cell patch-clamp in HEK293 cells and for five of the mutations previously associated with LQTS3, patch-clamp experiments showed an increased sustained sodium current suggesting an mechanistic overlap between LQTS3 and early-onset lone AF. In nine out of ten identified mutations and rare variants we observed compromised biophysical properties affecting the transient peak current.

**Conclusions** - In a cohort of early-onset lone AF patients we identified a high prevalence of SCN5A mutations previously associated with LQTS3. Functional investigations of the mutations revealed both compromised transient peak current and increased sustained current.

**Keywords**: atrial fibrillation; genes; long-QT syndrome; QT interval electrocardiography; SCN5A
Introduction

Atrial fibrillation (AF) is the most prevalent sustained cardiac arrhythmia, affecting almost seven million patients in the European Union and USA combined.\(^1\)\(^-\)\(^4\) In most cases AF arises secondary to hypertension, ischemic-, and/or structural heart disease relatively late in life.\(^1\)\(^,\)\(^5\) However, 10-20% of patients suffering from AF are younger than 60 years of age and free of traditional predisposing conditions. These patients are said to have lone AF. The mechanisms underlying lone AF are not fully understood, but interplay between multiple substrates and triggers are thought to constitute the etiology of AF.\(^6\) As such, early-onset lone AF has been suggested to be a primary electrical disease caused by disturbances in ion channel function.\(^6\)

Familial predisposition for AF has recently been recognized. Fox and colleagues showed that the development of AF in offspring is associated with parental AF.\(^8\) The importance of common genetic variants in the development of AF has been revealed in recent genome-wide association studies.\(^9\) Rare mutations in genes encoding potassium channels (\(KCNQ1, KCNH2, KCNA5, KCNJ2,5, KCNE1,2,3,5\)), sodium channels (\(SCN5A, SCN1-3B\)), a peptide hormone (\(ANP\)), a gap junction protein (\(GJA5\)) and a nuclear membrane protein (\(LMNA\)), have been linked to AF.\(^10\)\(^-\)\(^14\)

Mutations in \(SCN5A\), the gene encoding the \(\alpha\)-subunit underlying the dominant cardiac sodium current, is composed of a central pore-forming \(\alpha\)-subunit, \(NaV1.5\),\(^15\)\(^,\)\(^16\) and two \(\beta\)-subunits of the \(NaV\beta\) type. Besides its implication in Brugada syndrome (BrS), Long QT syndrome type 3 (LQTS3), and conduction defects, \(NaV1.5\) has recently been shown to play a role in AF.\(^17\) However, functional characterizations of \(SCN5A\) mutations associated with AF have been sparse. Two studies reported mutations that increased the transient peak current but showed no effect on the sustained current.\(^18\)\(^,\)\(^19\) Another study described a mutation with decreased transient peak
current. In a third study of a family affected by a LQTS3 mutation resulting in increased sustained sodium current, some members also had AF, suggesting that an increased sustained current could also play a role in AF. A very recent study of a human LQTS3 mutation expressed in a knock-in mouse model also suggested that the sustained sodium current could play a role in lone AF.

We hypothesised that early-onset lone AF patients would carry a high prevalence of \textit{SCN5A} mutations, as such individuals are particular likely to have a primary genetic defect as a substrate for AF. Also, we aimed to characterize possible mutations electrophysiologically by a patch-clamp approach to elucidate the functional impact of these mutations as such experiments have not previously been performed systematically.

**Methods**

**Study Subjects**

Patients were recruited from cardiology departments in eight hospitals in the Copenhagen region of Denmark. Patient records from all in- and outpatient activity in the past 10 years with the diagnose code (ICD-10) I48.9 (AF and atrial flutter) were collected. Only Patients with onset of lone AF before the age of 40 years (i.e, absence of clinical or echocardiograph findings of other cardiovascular diseases, hypertension, metabolic or pulmonary diseases) were included (Table 1). All patients were interviewed about family history of arrhythmia. Patients that carried mutations had a second interview specifically about family history of arrhythmia, sudden death, dilated cardiomyopathy, and other \textit{SCN5A} associated disease. All mutation carriers were offered a flecainide provocation test (2 mg/kg flecainide intravenously) to exclude BrS.

To distinguish between common genetic polymorphisms, rare variants, and mutations a group of ECG documented healthy controls without cardiac symptoms were collected.
The study conforms to the principles outlined in the Declaration of Helsinki and was approved by the Scientific Ethics Committee (reference number KF 01313322). All included patients gave written informed consent.

**Mutation screening**

Genomic DNA was isolated from blood samples using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). The entire coding sequence and splice junctions of SCN5A (NM_198056.2) (primers and PCR conditions are available on request) were amplified and analyzed using a high-resolution melting curve analysis as previously described (Light Scanner, Idaho Technology, UT, USA). Fragments with melting curves differing from the curves of wild-type DNA were purified and directly sequenced using Big Dye chemistry (DNA analyzer 3730, Applied Biosystems, CA, USA).

All identified non-synonymous variants were validated by resequencing in an independent PCR. DNA from twenty-two of the patients was sequenced directly using Big Dye chemistry. The group of healthy controls was screened employing high resolution melting curve analysis with probands included as positive controls. In probands with non-synonymous variants bidirectional sequencing of SCN1-3B (NM_001037.4, NM_004588, NM_018400.3), KCNQ1 (NM_000218.2), KCNH2 (NM_000238), KCNN3 (NM_002249.5), KCNA5 (NM_002234.2), KCNE1/2/3/5 (NM_001127668, NM_172201, NM_005472.4, NM_012282.2), KCNJ2,5 (NM_000891.2,NM_000890.3), KCNN3 [NM_002249.4]24 ANP (NM_006172.3), and LMNA (NM_005572) was performed.

**Bioinformatics**

We performed species alignment (Figure 1) and Polyphen-2 prediction analyses of variants.25 dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/) was searched for identified mutations and
variants.

The NHLBI GO Exome Sequencing Project (ESP) holds information on exome data from
next-generation sequencing of all protein-coding regions in 3510 persons of European American
ancestry.\textsuperscript{26} We compared the proportion of mutations or rare variants in SCN5A with minor allele
frequency (MAF) < 0.1\% in ESP with those variants in the lone AF population also with a MAF
< 0.1\% in ESP, assuming genetic homogenicity. We also compared the two populations with
regards to the proportion of previously published LQTS-associated variants, as reported in a
recent comprehensive review by Hedley et al\textsuperscript{27} and in data from the The Human Gene Mutation
Database (HGMD).\textsuperscript{28}

\textit{In vitro electrophysiology}

Mutations were introduced into Nav1.5 cDNA cloned in pcDNA3.1 (Invitrogen, Nærum,
Denmark), using standard mutated oligonucleotide extension PCR. All constructs were verified
by DNA sequencing. For patch-clamp studies HEK293 cells were transiently co-transfected with
0.3 \textmu g pcDNA3-hNav1.5 (wild-type (WT) or mutants), and 0.2 \textmu g of pcDNA3-eGFP as a
reporter gene, using Lipofectamine and Plus reagent (Invitrogen) according to the manufacturer’s
instructions. Patch-clamp experiments were performed at room temperature (20–22°C) 2-3 days
after transfection. Patch-clamp recordings were conducted using an internal solution containing
(mmol/L) CsCl 60; CsAspartate 70; EGTA 11; MgCl\textsubscript{2} 1; CaCl\textsubscript{2} 1; HEPES 10; and Na\textsubscript{2}-ATP 5,
pH 7.2 with CsOH; external solution NaCl 130; CaCl\textsubscript{2} 2; MgCl\textsubscript{2} 1.2; CsCl 5; HEPES 10; and
glucose 5, pH 7.4 with CsOH. Data analyses were done as previously described.\textsuperscript{29}
A potential increase in sustained current was investigated by applying 30 \textmu M tetrodotoxin (TTX)
for the mutations R1626H and D1819N.

Data Analysis of Electrophysiological Experiments
Peak current densities were measured during an activation protocol and $I_{Na}$ densities (pA/pF) were obtained by dividing peak $I_{Na}$ by cell capacitance. For activation and steady-state inactivation curves, data from individual cells were fitted with a Boltzmann equation, $y(V_m) = 1/[1 + \exp((V_m-V_{1/2})/K)]$, in which $y$ is the normalized current or conductance, $V_m$ the membrane potential, $V_{1/2}$ the voltage at which half of the channels are activated or inactivated, and $K$ the slope factor. The decay characteristics of the fast transient current was fitted best with time constants using the following equation: $I = I_0 + A \exp(-t/T)$, where $t$ is the time from the beginning of the test pulse and $T$ is the time constant of current decay. Recovery curves from inactivation were obtained by giving a 50 ms, -20 mV depolarizing pulse followed clamping to four different pre-potentials. Recovery was fitted with mono-exponential function:

$I_{test}/I_{pre} = Y_0 + A \exp^{-T/T}$, where $Y_0$ is the offset, $A$ is amplitude, and $T$ is the time constant. Data are presented as mean±SEM unless otherwise noted. Student’s unpaired t-test, one-way ANOVA, or Fisher’s exact tests were used to test for significant differences. Normal distribution of the data set was tested by Shapiro-Wilk normality test using GraphPadPrism 5.0 software. A value of $P<0.05$ was considered statistically significant. The authors had full access to the data and take responsibility for its integrity.

Results

Study cohort

The study population consisted of 192 patients with onset of AF ranging from 16 to 39 years without any concomitant disease. All included individuals were of Danish/Caucasian ethnicity. Clinical data is shown in Table 1.30

Mutation screening

Screening of SCN5A in the 192 lone AF patients revealed ten non-synonymous mutations,
S216L, T220I, R340Q, T1304M, F1596I, R1626H, D1819N, R1897W, V1951M and F2004L (Table 2), five had not been functionally characterized before (see below). S216L was found in two lone AF patients. S216L and F2004L have previously been described in healthy controls (both with a MAF = 0.09%) and were therefore confided as rare variants in the following. In addition, T220I, T1304M, R1897W were identified with very low frequency in the ESP however, the disease status of these individuals was unknown.

None of the mutations were present in our control population (n=216) and have not previously been reported in another large group of healthy controls (n=1100). All patients were heterozygous for the mutations.

All patients carrying non-synonymous mutations were subsequently screened for mutations in the whole coding region of the genes already known to be associated with AF: SCN1-3B; KCNQ1, KCNH2, KCNA5; KCNJ2-3; KCNJ5, KCNN3; KCNE1,2,3,5, ANP; and LMNA, but no additional mutations were found.

**Bioinformatics**

All mutations were found to be highly conserved across species except for V1951M and F2004L which are not conserved and R340Q which was conserved only in eutherian mammals (Figure 1). A PolyPhen2 prediction indicated that seven out the ten mutations and rare variants in SCN5A were predicted to be probably or at least possibly damaging (Table 2). We identified a significantly higher frequency of rare SCN5A variants (MAF<0.1%) in the lone AF patients when compared to the frequencies reported in ESP (MAF 2.9% vs. 1.1%, P = 0.013). This was also the case with regards to SCN5A variants previously associated with LQTS3 (MAF 1.6% vs. 0.3%, P = 0.003).

**Family history**

All patients carrying a mutation in SCN5A were interviewed specifically about family history of
arrhythmias and SCN5A related diseases and five of the probands had a family history of arrhythmia. The proband carrying the mutation F1596I had a mother and a sister who were both affected by AF, but as they were both deceased genetic screening was not possible. The proband carrying D1819N also had a family history of AF, but without cosegregation of the mutation. The proband carrying R1897W had a mother diagnosed with post-operative AF at high age but she did not carry the mutation. The patient carrying the mutation V1951M had a father diagnosed with non-sustained VT, though he was not a mutation carrier either. The proband carrying F2004L had a father with AF, but he was unavailable for genetic testing. For the other patients, there was no family history of AF, and hence no genetic testing of relatives was performed.

**In vitro electrophysiology**

SCN5A mutations not previously characterized electrophysiologically (R340Q, R1626H, D1819N, R1897W, and V1951M) were investigated for a potential functional impact. We expressed wild-type or mutant channels in mammalian HEK293 cells and addressed electrophysiological parameters by whole-cell patch-clamp experiments (Figure 2 and Table 3). No significant difference in peak current density was observed in any of the mutants as compared to control. However, differences were observed in steady-state activation and in several different inactivation parameters summarized in Table 3. In brief, R340Q showed a negative voltage-shift of both steady-state activation and inactivation together with a reduced time constant for onset (decay) of fast inactivation (Figure 2D, E, B, respectively). R1626H gave a positive voltage-shift of steady-state activation and a negative voltage-shift of steady-state inactivation together with a decreased onset of fast inactivation (Figure 2D, E, B, respectively). D1819N revealed a minor change in the onset of fast inactivation parameters with an increase of the decaying time constant at depolarizing potentials (Figure 2B). R1897W showed a drastic
negative voltage-shift of the steady-state inactivation potential (Figure 2E), and V1951M gave a decrease of the time-dependent inactivation at different potentials as well as a decrease of onset of inactivation time constant (Figure 2B, F, respectively).

The R1626H mutant had a moderately increased sustained current component, while D1819 produced pronounced sustained sodium currents (Figure 3B-D).

ECG

Flecainide provocation tests did not induce Brugada ECG patterns in any of the tested probands (Table 4). Five out of ten of the identified mutations and rare variants (S216L, R340Q, T1304M, D1819N, and V1951M) have previously been associated with LQT3 syndrome. One of the two patients carrying S216L had a borderline prolonged QTc interval of 469 ms whereas the other proband, who was also a carrier of H558R, a variant previously shown to be able to rescue other mutations functionally, had a QTc within the normal range of 438 ms.

At baseline the patient harboring the R1626H mutation had a 443 ms QTc interval, but interestingly, during flecainide testing the QTc interval increased to 495 ms. The patient carrying D1819N had a borderline prolonged QTc interval of 467 ms. This patient also had a relative large 43 ms increase in QTc interval during the flecainide test. The V1951M proband had a QTc of 425 ms at baseline, but this patient also displayed a relatively large increase in QTc of 41 ms during flecainide testing. Both patients carrying R340Q or T1304M, respectively, had normal QTc intervals (Table 4).

Discussion

This study is the first comprehensive attempt to associate early-onset lone AF with mutations in SCN5A. In a cohort of 192 patients with onset of lone AF before the age of 40 years, we identified eight mutations (T220I, R340Q, T1304M, F1596I, R1626H, D1819N, R1897W,
V1951M,) in SCN5A. The high degree of conservation across species indicates that these residues are important for channel function. We also identified two rare SCN5A variants (S216L in two probands and F2004 in one proband). Six out of eleven SCN5A positive probands (3.2% of the total population) carried a mutation or rare variant previously associated with LQT3 syndrome.

Genetic screening of the lone AF patients revealed a much higher prevalence of mutations or rare variants in SCN5A as expected from the prevalences in ESP (MAF 2.9% vs. 1.1%, P = 0.013), representing the general population. Also mutations or rare variants previously associated with LQTS3 were present with a higher prevalence in the lone AF patients as compared to ESP (MAF 1.6% vs. 0.3%, P = 0.003). Despite some limitations in comparing MAF in the two populations (being different screening techniques, no possibility of matching on age and gender, and different geographic regions), this quantitative approach strongly supports the hypothesis that the present SCN5A mutations or rare variants identified in the lone AF patients might be involved in the pathogenesis of AF.

All mutation carriers had a QTc interval within the normal range (<470 ms), however two probands had a borderline prolonged QTc interval of 467 ms and 469 ms, respectively. Noteworthy, it has recently been shown that individuals carrying a LQTS associated mutation with a QTc interval within the normal range (<440 ms) also have an increased risk for life-threatening cardiac events.38 Hence, we speculate that the lone AF patients in our cohort carrying mutations previously associated with LQTS3 may have an increased risk of life-threatening arrhythmias. If lone AF patients in general carry a high prevalence of LQTS3 associated variants, then as a group, they might have an increased risk of life-threatening arrhythmias. This novel finding could have potentially clinically implications for future risk stratification in lone AF
patients. However, further investigations are warranted in order to address a potential benefit of genetic screening of lone AF patients in a clinical setting. Interestingly, in the present group of $SCN5A$ genotype-positive patients, those patients carrying a LQTS3 associated variant also presented with the longest QTc intervals. The two patients with the shortest QTc intervals were the only two $SCN5A$ positive probands who carried the variant R558H (Table 4), which has been shown able to rescue a number of other $SCN5A$ variants (Table 2).$^{37,39}$

Our results from the present cohort of early-onset lone AF patients indicate that $SCN5A$ mutations only in very rare cases give rise to highly penetrant monogenic forms of AF (Table 2). This is in contrast to a study by Darbar et al. who reported a number of $SCN5A$ mutations that cosegregated with familial AF.$^{35}$ We envision several explanations for this discrepancy. Firstly, the two cohorts differed in gender, age and size of the families. Secondly, due to the relative age of the probands’ parents, they may not have developed AF yet although being predisposed for this. Thirdly, a cohort selection bias may exist, in that patients with familial aggregation potentially more often could have been referred to the cohort described by Darbar et al. (Mayo Clinic, US). The proband carrying R1897W had a mother diagnosed with post-operative AF at high age and she did not carry the mutation. AF after surgery is very common. The patient carrying the mutation V1951M had a father diagnosed with non-sustained VT, though he was not a mutation carrier either. A recent paper gave important input to the discussion about reduced penetrance. In LQTS type 1 patients, it was shown that variants in the 3’UTR-region of the $KCNQ1$ gene modify disease severity in an allele-specific manner$^{40}$ and this mechanism may also be important in other LQTS genes such as $SCN5A$. The lack of familial cosegregation in combination with the fact that both rare $SCN5A$ variants and previously LQTS3 associated variants are highly overrepresented in the lone AF patients as compared to the general
population, points in the direction that these variants might be important disease modifiers rather than monogenic causes of lone AF.

**Compromised peak sodium current**

To investigate whether the mutations reported here are disease-causing, whole-cell patch-clamp electrophysiological investigations were performed. We analysed five *SCN5A* mutations that have not been functionally investigated previously, namely R340Q, R1626H, D1819N, R1897W, and V1951M. None of the mutations had a significant effect on peak current density. Steady-state activation was found altered for two mutations. Channels harbouring the R340Q mutation showed a negative potential shift, which means that these channels open at more negative membrane potentials, increasing the availability of the channels (Figure 2 and Table 2). R1626H channels showed a positive voltage-shift of activation which is expected to reduce channel availability. Since Na\textsubscript{v}1.5 channels are inactivated at potentials close to the resting membrane potential of cardiomyocytes, small changes in steady-state inactivation are expected to give large impact on sodium channel availability. Indeed, for the R340Q, R1626H, and R1897W mutations, we observed a more than 5 mV negative shift in steady-state inactivation (Figure 2, Table 3). We also investigated the onset (or decay) of inactivation which is a measure of the width of the sodium peak. If the onset of inactivation time constant is decreased the channel will close faster, resulting in a decreased depolarizing power of the mutated channel. Decreased time constants were found for R340Q and V1951M. A third inactivation property is the time-dependent recovery from inactivation. The V1951M mutation was found to have shorter recovery time at all tested potentials. A faster recovery could be speculated to be pro-arrhythmic as the sodium channel complexes are released from inactivation earlier and can thereby contribute to wave-length shortening.\textsuperscript{17} In conclusion, all five mutations displayed altered phenotypes compared to
wild-type Na\textsubscript{v}1.5. Most of the observed changes pointed towards a decreased transient peak current. Of those mutations previously been studied by others, T220I\textsuperscript{34} displayed decreased peak current, while S216L\textsuperscript{32}, T1304M\textsuperscript{32} and L2004F\textsuperscript{32} showed electrophysiological parameters resulting in increased availability of the sodium channels in the early (transient) part of the action potential (Table 2). F1596I has been shown not to affect any of the peak current parameters and its role in AF is therefore questionable (Table 2).\textsuperscript{7} Our results together with other available data support the notion that both an increase\textsuperscript{18,19} and a decrease in the transient sodium peak current predisposes for AF.\textsuperscript{34}

Pappone and colleagues have recently reported that in six percent of a lone AF population a BrS type 1 ECG pattern could be induced by flecainide testing.\textsuperscript{41} Flecainide testing of our SCN5A positive probands did not reveal any BrS type 1 ECG pattern, indicating that these subjects are unlikely to have a concealed BrS.

**Increased sustained sodium current**

Flecainide has, apart from blocking I\textsubscript{Na}, also been shown to block the Kv11.1 (hERG1) potassium channel, which is responsible for the fast delayed rectifier current, I\textsubscript{Kr}, and can therefore potentially unmask increased sustained sodium current.\textsuperscript{42} It can be speculated that the six probands carrying a SCN5A mutation previously associated with LQTS3 are predisposed for AF through increased sustained sodium current, a mechanism thought to at least partly underlie LQTS3. Indeed, for three (S216L, T1304M, and L2004F) out of the six mutations increased sustained sodium current has been reported (8-, 7- and 4-fold, respectively).\textsuperscript{32} In addition, three out of seven patients carrying a mutation previously associated with LQTS3 had either a borderline prolonged QT\textsubscript{c} interval at baseline or a higher increase in QT\textsubscript{c} interval during flecainide test than the expected value of 21±17 ms for healthy individuals (Table 4).\textsuperscript{43}
In our cohort, the patient carrying the novel R1626H mutation had an unexpectedly large increase in QTc (52 ms) during flecainide test and the patient carrying D1819N had a borderline prolonged QTc interval of 467 ms at baseline. Hence, we investigated the sustained sodium current for R1626H and D1819N. Patch-clamp experiments revealed a 2-3 fold increase in sustained current for the R1626H mutation while the D1819N conducts a dramatically 6-10 fold increased sustained current. Hence, the in vitro investigations confirmed the effect of flecainide on the QTc interval observed in the two probands, indicating that the sustained component of the sodium current might play a role in the pathogenesis of AF. This is in line with a study employing isolated atrial myocytes from AF patients which showed increased sustained sodium current.44 Further, Lemoine et al. have very recently shown atrial action potential prolongation, atrial early after depolarisations (EAD), and triggered activity in a genetically modified animal model of human LQTS3. Treatment with ranolazine, a blocker of the sustained sodium currents, normalised the number of EAD’s in this model.22 Of note, patients treated with ranolazine for angina pectoris had a lower incidence of supraventricular tachycardias.45

Our study on human AF together with the mice experiments by Lemoine et al. for the first time indicate a possible overlap between the mechanisms underlying LQTS3 and lone AF with action potential prolongation as a substrate and EADs as triggers for arrhythmia. However, both decreased and increased transient peak sodium current have also been suggested as possible mechanisms for AF;18,19, 20 and further studies are needed to reveal the electrophysiological mechanisms behind AF.

**Limitations**

We only analyzed the coding regions of SCN5A, and mutations occurring in gene regions other than coding regions cannot be excluded. We used a conventional heterologous expression
system, which differs from native cardiomyocytes. Furthermore, for the electrophysiological parameters investigated several changes of a number parameters were observed. Although these data provide strong support for discussing whether a given mutation is preferentially a loss- or gain-of-function mutation they cannot be regarded as conclusive.

**Conclusion**

We identified eight mutations and two rare variants in SCN5A in 192 patients with early-onset lone AF. A high number of these lone AF patients carried a mutation or rare variant previously associated with LQTS3 as compared to the expected frequency in the general population (MAF 1.6% vs. 0.3%, P = 0.003). All identified variants have been investigated electrophysiologically and in nine of them compromised peak sodium current was found, while five variants showed increased sustained sodium current. Our results thereby indicate that both gain- and loss-of-function alterations in the electrophysiological properties of the cardiac sodium current may lead to the development of AF in young adults.

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**Conflict of Interest Disclosures:** None.

**References:**


Modify Disease Severity in Patients with Type 1 Long QT Syndrome in an Allele-Specific Manner. *Eur Heart J.* 2012;33:714-723.


**Table 1.** Clinical characteristics of the lone AF population (n=192)

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<td>Median age of onset, y (IQR)</td>
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<tr>
<td>Male gender, %</td>
<td>82</td>
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<tr>
<td>Height, cm</td>
<td>183±9</td>
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<td>Weight, kg</td>
<td>89±17</td>
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<td>BMI, kg/m²</td>
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<td>Family history of AF</td>
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<td>1st degree relatives with AF, %</td>
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All numbers are reported as mean ± standard-deviation unless otherwise noted. IQR; Interquartile range.
### Table 2 Summary of Na\(_{\text{v}}\)1.5 sodium channel mutations and rare variants in SCN5A.

<table>
<thead>
<tr>
<th>Amino Acid change</th>
<th>Nucleotide change</th>
<th>Frequencies in patients</th>
<th>Conserv.</th>
<th>Exon</th>
<th>Location</th>
<th>Polypen -2 score</th>
<th>Reported in</th>
<th>Function</th>
</tr>
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<tbody>
<tr>
<td>S216L</td>
<td>c.647C&gt;T</td>
<td>2/192</td>
<td>HC</td>
<td>6</td>
<td>Transmembrane, Voltage-sensor</td>
<td>benign 0.000</td>
<td>LQT/(^{32})SIDS/(^{32})</td>
<td>Positive voltage-shift in steady-state inactivation; Increase in fast inactivation; Negative voltage-shift in recovery from inactivation; 8-fold increased sustained sodium current/(^{32})</td>
</tr>
<tr>
<td>T220I</td>
<td>c.659 C&gt;T</td>
<td>1/192</td>
<td>HC</td>
<td>6</td>
<td>Transmembrane, Helical; Voltage-sensor</td>
<td>probably damaging 0.97</td>
<td>AF/(^{32}) SS/(^{32})</td>
<td>Decreased peak current; Negative voltage-shift in steady-state inactivation; Slower time-dependent recovery from inactivation/(^{32})</td>
</tr>
<tr>
<td>R340Q</td>
<td>c.1018 C&gt;G</td>
<td>1/192</td>
<td>CM</td>
<td>8</td>
<td>Extracellular</td>
<td>possibly damaging 0.93</td>
<td>LQTF/(^{32}) SIDS/(^{32})</td>
<td>Negative voltage-shift in steady-state activation; Negative voltage-shift in steady-state inactivation; Increased fast inactivation</td>
</tr>
<tr>
<td>T1304M</td>
<td>c.3911C&gt;T</td>
<td>1/192</td>
<td>HC</td>
<td>22</td>
<td>Transmembrane, Voltage-sensor</td>
<td>probably damaging 1.00</td>
<td>LQT/(^{32}) SIDS/(^{32})</td>
<td>Positive voltage-shift in steady-state activation; Positive voltage-shift in steady-state inactivation; Increased fast inactivation; Faster time-dependent recovery from inactivation; 7-fold increased sustained sodium current/(^{32})</td>
</tr>
<tr>
<td>F1596I</td>
<td>c.4786 T&gt;A</td>
<td>1/192</td>
<td>HC</td>
<td>26</td>
<td>Transmembrane, Helical</td>
<td>probably damaging 0.96</td>
<td>Ab</td>
<td>Normal peak current parameter, sustained current not investigated/(^{7})</td>
</tr>
<tr>
<td>R1626H</td>
<td>c.4877 G&gt;A</td>
<td>1/192</td>
<td>HC</td>
<td>28</td>
<td>Transmembrane, Helical; Voltage-sensor</td>
<td>probably damaging 1.00</td>
<td>Novel</td>
<td>Positive voltage-shift in steady-state activation; Negative voltage-shift in steady-state inactivation; Decreased fast inactivation 2-3-fold increased sustained sodium current</td>
</tr>
<tr>
<td>D1819N</td>
<td>c.5455 G&gt;A</td>
<td>1/192</td>
<td>HC</td>
<td>28</td>
<td>Intracellular</td>
<td>probably damaging 0.99</td>
<td>LQT/(^{32})</td>
<td>Negative voltage-shift in steady-state inactivation; Decreased fast inactivation; 6-10-fold increased sustained sodium current</td>
</tr>
<tr>
<td>R1897W</td>
<td>c.5689 C&gt;T</td>
<td>1/192</td>
<td>HC</td>
<td>28</td>
<td>Intracellular</td>
<td>probably damaging 1.00</td>
<td>novel</td>
<td>Negative voltage-shift in steady-state inactivation</td>
</tr>
<tr>
<td>V1951M</td>
<td>c.5851 G&gt;A</td>
<td>1/192</td>
<td>NC</td>
<td>28</td>
<td>Intracellular</td>
<td>benign 0.000</td>
<td>AF/(^{32})</td>
<td>Decreased fast inactivation; Positive voltage-shift in recovery from inactivation</td>
</tr>
<tr>
<td>L2004F</td>
<td>c.5851 G&gt;A</td>
<td>1/192</td>
<td>NC</td>
<td>28</td>
<td>Intracellular</td>
<td>benign 0.000</td>
<td>SIDS/(^{32})</td>
<td>Positive voltage-shift in steady-state inactivation; Decreases fast inactivation; Faster recovery from inactivation; 4-fold increased sustained sodium current/(^{32})</td>
</tr>
</tbody>
</table>

Conserv. = degree of conservation for the mutated site among multiple species: CM = conserved among large mammals; HC = highly conserved; NC = not conserved.
<table>
<thead>
<tr>
<th></th>
<th>Peak current at -15 mV (pA/pF)</th>
<th>n</th>
<th>Steady-state activation V_{1/2} (mV)</th>
<th>Slope k value</th>
<th>n</th>
<th>Steady-state inactivation V_{1/2} (mV)</th>
<th>Slope k value</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>-539± 55</td>
<td>23</td>
<td>-27.9± 1.3</td>
<td>6.6± 0.3</td>
<td>22</td>
<td>-85.6± 0.9</td>
<td>5.5± 0.1</td>
<td>25</td>
</tr>
<tr>
<td>R340Q</td>
<td>-462± 90</td>
<td>14</td>
<td>-34.1± 1.5**</td>
<td>6.3± 0.5</td>
<td>14</td>
<td>-91.1± 1.3**</td>
<td>5.7± 0.2</td>
<td>14</td>
</tr>
<tr>
<td>R1626H</td>
<td>-380± 74</td>
<td>15</td>
<td>-23.5± 1.8*</td>
<td>7.6± 0.4*</td>
<td>14</td>
<td>-91.2± 1.9**</td>
<td>5.6± 0.2*</td>
<td>13</td>
</tr>
<tr>
<td>D1819N</td>
<td>-385± 59</td>
<td>15</td>
<td>-27.7± 1.8</td>
<td>7.5± 0.6</td>
<td>14</td>
<td>-85± 0.5</td>
<td>5.9± 0.2*</td>
<td>14</td>
</tr>
<tr>
<td>R1897W</td>
<td>-465± 65</td>
<td>15</td>
<td>-29.5± 1.6</td>
<td>7.2± 0.5</td>
<td>15</td>
<td>-91.8± 1.5**</td>
<td>5.5± 0.2</td>
<td>14</td>
</tr>
<tr>
<td>V1951M</td>
<td>-616± 108</td>
<td>10</td>
<td>-31.5± 1.8</td>
<td>6.9± 0.4</td>
<td>10</td>
<td>-85.6± 1.0</td>
<td>5.6± 0.2</td>
<td>10</td>
</tr>
</tbody>
</table>

*) Significantly different from Na\textsubscript{v}1.5-WT: *)p < 0.05; **)p < 0.01; ***)p < 0.001. n is the number of experiments.
Table 4. Clinical characteristics of probands with SCN5A variants

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Genotype</th>
<th>H558R genotype</th>
<th>Gender</th>
<th>Phenotype</th>
<th>Onset, y</th>
<th>ECG at inclusion</th>
<th>ECG during flecaïnide test</th>
<th>Flecaïnide test</th>
<th>Family history of AF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S216L</td>
<td>HR</td>
<td>M</td>
<td>Persistent</td>
<td>36</td>
<td>Normal (QTc 438ms)</td>
<td>-</td>
<td>Not done</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>S216L</td>
<td>HH</td>
<td>M</td>
<td>Persistent</td>
<td>39</td>
<td>Normal (QTc 469ms)</td>
<td>-</td>
<td>Not done</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>T220I</td>
<td>HH</td>
<td>M</td>
<td>Paroxystic</td>
<td>35</td>
<td>Normal (QTc 422ms)</td>
<td>-</td>
<td>Not done</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>R340Q</td>
<td>HH</td>
<td>M</td>
<td>Paroxystic</td>
<td>26</td>
<td>Normal (QTc 422ms)</td>
<td>Not available</td>
<td>Negative</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>T1304M</td>
<td>HH</td>
<td>M</td>
<td>Paroxystic</td>
<td>37</td>
<td>r'-wave in V1/V2 (QTc 439ms)</td>
<td>QRS increase 28 ms QTc increase 30 ms</td>
<td>Negative</td>
<td>Mother and mother’s sister with AF, both decreased</td>
</tr>
<tr>
<td>6</td>
<td>F1596I</td>
<td>HH</td>
<td>M</td>
<td>Persistent</td>
<td>39</td>
<td>Low voltage (QTc 428)</td>
<td>QRS increase 15 ms QTc increase 19 ms</td>
<td>Negative</td>
<td>None</td>
</tr>
<tr>
<td>7</td>
<td>R1626H</td>
<td>HH</td>
<td>M</td>
<td>Paroxystic</td>
<td>37</td>
<td>Normal (QTc 443ms)</td>
<td>QRS increase 26 ms QTc increase 52 ms</td>
<td>Negative</td>
<td>None</td>
</tr>
<tr>
<td>8</td>
<td>D1819N</td>
<td>HH</td>
<td>F</td>
<td>Paroxystic</td>
<td>25</td>
<td>Short PR of 106ms (QTc 467ms)</td>
<td>QRS increase 4 ms QTc increase 48 ms</td>
<td>Negative</td>
<td>Family history of AF, but no mutation cosegregation</td>
</tr>
<tr>
<td>9</td>
<td>R1897W</td>
<td>HH</td>
<td>M</td>
<td>Paroxystic</td>
<td>38</td>
<td>r'-wave and J-point elevation in V1/V2 (QTc 397)</td>
<td>QRS increase 20 ms QTc increase 16 ms</td>
<td>Negative</td>
<td>Mother with postoperative AF, no mutation</td>
</tr>
<tr>
<td>10</td>
<td>V1951M</td>
<td>HR</td>
<td>M</td>
<td>Persistent</td>
<td>22</td>
<td>J-wave in II, III and aVF (QTc 425ms)</td>
<td>QRS increase 18 ms QTc increase 41 ms</td>
<td>Negative</td>
<td>Father diagnosed with VT, no mutation</td>
</tr>
<tr>
<td>11</td>
<td>F2004L</td>
<td>HR</td>
<td>M</td>
<td>Chronic</td>
<td>38</td>
<td>AF</td>
<td>-</td>
<td>Not done</td>
<td>Father AF, not available for genetic testing</td>
</tr>
</tbody>
</table>
Figure Legends:

Figure 1. A. DNA sequencing traces (chromatograms) for variants identified in SCN5A. B. Evolutionary conservation between species. The location of mutated amino acid is marked in red. C. Position of the mutations indicated in schematic of protein topology.

Figure 2 Electrophysiological characterization of SCN5A mutants. A. Representative current traces obtained with a current/voltage protocol (inset in D) for wild-type (WT) and the five Na\textsubscript{V}1.5 mutations. B. Onset of fast inactivation. Single exponential fit to the decaying phase of the current traces (as shown in A). C. Current/voltage relationship of WT and Na\textsubscript{V}1.5 mutants. D. Steady-state activation curves. Activation properties were determined from I/V relationships by normalizing peak I\textsubscript{Na} to driving force and maximal I\textsubscript{Na}, and plotting normalized conductance vs. mV. E. Steady-state inactivation curves. Boltzmann curves were fitted to both steady-state activation and inactivation data. F. Time- and voltage-dependent recovery from inactivation. The time-dependent recovery from inactivation at different voltage potentials (inset) was fitted with a mono-exponential relationship and the tau values plotted. (A,C-E) Averaged values and the numbers of cells measured are presented in Table 3. (B,F) n = 10 for each group. *)P<0.05, **)P<0.01, and ***)P<0.001. ● WT, ◊ R340Q, ▼ R1626H, △ R1897W, ○ D1819N, □ V1951M. Error bars represent the mean ±SEM. In some figures, the standard error bars are smaller than the data symbols.

Figure 3 Sustained sodium current (I\textsubscript{NaL}) of R1626H and D1819N is increased at different voltages. Representative recordings in absence or presence of TTX for WT (n= 6)(A), R1626H
(n=6)(B), and D1819N (n=6)(C). Currents were activated by a 500 ms step to −20 mV from a holding potential of −100 mV. For comparison, the peak and late current are shown at different scales. The sustained currents were normalized to the peak current observed in each trace. (D) Summarized data of INaL at different voltages. Currents were activated by a 500 ms step from −30 mV to 0 mV in 10 mV increments from a holding potential of −100 mV. Currents in presence of TTX were subtracted from currents recorded in the absence of TTX to determine the TTX-sensitive current. INaL was measured as mean current between 450-500 ms and the ratio between the TTX-sensitive peak and late current was calculated for WT, R1626H and D1819N. At each condition, the difference in INaL between R1626H or D1819N and WT was significant. *)P<0.05, **)P<0.01, and ***)P<0.001.
A) S216L  T220I  R340Q  T1304M  F1596I  
   GTCTCAGCC  CGACCATTC  TAACCGTG  CAGATG  CGACTCTCGT  ATCCAG 
   TTAATC  AATATT 

R1626H  D1819N  R1897W  V1951M  F2004L  
   ATCCGGCTG  AGCTGACTG  ACGCTG  AGCTGATG  CGACTCTCCC  ATCCTC 
   CACCAC  GATGAC 

B) | S216 | T220 | R340 | T1304 | F1596 | R1626 | D1819 | R1897 | V1951 | F2004 |
---|------|------|------|------|-------|-------|-------|-------|-------|-------|
Human | NVRSL | LRTFR | GYRCL | LRTLR | PDFVV | VIRLA | PAMAL | TLRKX | AYVMS | ADFFP |
Rat   | NVRSL | LRTFR | GYRCL | LRTLR | PDFVV | VIRLA | PAMAL | TLRKX | AYVMS | ADFFP |
Mouse | NLRSL | LRTFR | GYRCL | LRTLR | PDFVV | VIRLA | PAMAL | TLRKX | AYMN  | ADFFP |
Cow   | NVRSL | LRTFR | GYRCL | LRTLR | PDFVV | VIRLA | PAMAL | TLRKX | AYMN  | ADFFP |
Dog   | NVRSL | LRTFR | GYRCL | LRTLR | PDFVV | VIRLA | PAMAL | TLRKX | AYTM  | ADLPP |
Chicken | NVRSL | LRTFR | GYRCL | LRTLR | PDFVV | VIRLA | PAMAL | TLRKX | AFVN  | TDCLL |

* **** * ***  **** ***  **** ***  **** ***  **** ***  **** ***  **** * **

C) ![Diagram of mutations](image)

- **S216L**
- **T220I**
- **R340Q**
- **T1304M**
- **R1626H**
- **F1596I**
- **D1819N**
- **R1897W**
- **V1951M**
- **F2004L**
High Prevalence of Long QT Syndrome Associated SCN5A Variants in Patients with Early-Onset Lone Atrial Fibrillation
Morten S. Olesen, Lei Yuan, Bo Liang, Anders G. Holst, Nikolaj Nielsen, Jonas B. Nielsen, Paula L. Hedley, Michael Christiansen, Søren-Peter Olesen, Stig Haunsø, Nicole Schmitt, Thomas Jespersen and Jesper H. Svendsen

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